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Increased susceptibility to microdamage in *Brtl/+* mouse model for osteogenesis imperfecta★

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Abstract

Osteogenesis imperfecta (OI) is a genetic disease of collagen or collagen-related proteins that adversely impacts bone mass and fracture resistance. Little is known regarding the role that microdamage plays in OI and whether or not OI bone is more prone to damage accumulation than bone with unaffected collagen. The *Brtl/+* mouse is a heterozygous model for OI which contains a Gly349Cys substitution in one *COL1A1* allele, and demonstrates a low ductility phenotype. At 8 weeks of age, *Brtl/+* demonstrates an increase in osteoclast number, which mimics the upregulated bone turnover often found in OI patients. We hypothesize that upregulated osteoclast activity in *Brtl/+* is due, in part, to increased remodeling associated with microdamage repair. In the present study, we used *Brtl/+* to investigate the susceptibility of OI bone to microdamage. The mouse ulnar loading model was used to induce microdamage and to test the hypothesis that *Brtl/+* is more susceptible to damage accumulation than age-matched wild type (WT) counterparts. Linear elastic fracture mechanics (LEFM) was used to investigate the fracture toughness properties of both *Brtl/+* and WT bones to determine if there is any correlation with toughness and the degree of microdamage.

Results show that *Brtl/+* ulnae subject to normal cage activity demonstrate an inherently larger amount of microdamage than WT controls. Following axial compressive loading, *Brtl/+* ulnae are more prone to damage than WT counterparts despite demonstrating a greater resistance to whole-bone deformation. Fracture toughness results demonstrate that *Brtl/+* specimens, despite not exhibiting a significant difference, display a trend toward lower fracture toughness values than their WT counterparts. Correlations show that microdamage levels tend to increase as fracture toughness decreases. Together, these findings may have strong clinical implications for explaining increased fragility and remodeling activity in OI patients.

Keywords

Microdamage; Osteogenesis imperfecta; Mouse model; Fracture toughness; Collagen

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Introduction

Osteogenesis imperfecta (OI) is a genetic disease that adversely impacts bone mass and fragility, and produces other connective tissue manifestations in bone. It typically affects young children [1], and the severity ranges from lethality in the perinatal stage to a barely detectable form of connective tissue disorder [2]. OI is identified in approximately 1 in 20,000 births and affects between 20,000 and 50,000 individuals in the United States [3]. Patients who suffer from OI typically have a mutation in either COL1A1 or COL1A2, which encodes the α chains of type I collagen [4]. Although most forms of OI are autosomal dominant, about 10% of cases have autosomal recessive inheritance of mutations in proteins responsible for post-translational modification of collagen, resulting in similar changes in the skeletal composition, structure and morphology to classical OI [5–7].

Studies have demonstrated a disproportionate up-regulation of osteoclast and osteoblast activities in OI patients, with an overall imbalance favoring osteoclast resorption [8,9]. The mechanical and physiologic mechanisms responsible for this phenomenon are not fully understood. Increased bone turnover and fragility inherent to OI may be related to targeted repair of elevated levels of bone microdamage resulting from the brittle properties of OI. However, there has been limited investigation on the presence and impact of microdamage in OI patients. Animal models representing OI suggest that OI bone may be more susceptible to microdamage accumulation during cyclic loading [10,11]. This study uses the Brittle IV (Brtl/+) mouse model for OI to determine the impact of an OI-causing collagen mutation on the generation and accumulation of bone microdamage. Brtl/+ has a Gly349→Cys (G349C) substitution in one *coll1a1* allele, reproducing the genetic mutation found in a Type IV OI patient [12]. Brtl/+ demonstrates bone fragility, a moderately deformed skeleton, and a low ductility phenotype, accurately representing the biomechanical phenotype of the disease [13]. Furthermore, Brtl/+ features an up-regulation of osteoclast and osteoblast activities consistent with observations found in OI patients [14]. The direct cause of this upregulation has yet to be described.

The primary goal of this study is to determine whether the Brtl/+ mouse is more susceptible to microdamage accumulation compared to wild type (WT) controls and to determine the correlation between fracture toughness and propensity to accumulate microdamage. We propose that a propensity to accumulate damage is directly correlated with reductions in fracture toughness found in Brtl/+. Understanding the role of this inherent bone matrix property will help elucidate the role of collagen mutations in governing susceptibility of OI bone to microdamage.

Materials and methods

Murine model

A total of 18 male Brtl/+ and wild type WT (n=9 each) mice were bred from our colony and used in this study. Brtl/+ mice have a mixed background of Sv129/CD-1/C57BL/6S and are bred by crossing heterozygous Brtl/+ with WT [12]. Mice were euthanized at 8 weeks of age in preparation for all loading experiments. All procedures used in this study were approved by the University Committee on Use and Care of Animals (UCUCA) at the University of Michigan.

In-situ strain measurements

The ulnar loading model [15] was used throughout this study to produce compressive loads and induce microdamage. This loading model has been shown to produce loads primarily within the ulna and not the radius, and has been shown to induce microcrack formation within the ulna of rats [16]. The right forearms of mice were exposed to cyclic uniaxial

compression loading across the olecranon and carpus process as described by others [16,17]. Loading was done using a custom built servo-motor loader. Force-controlled, haversine, cyclic loading was performed at 1 Hz frequency while force and strain voltages were recorded using Labview 7.1 software (National Instruments, U.S.A.).

Six cadaver mice (n=3 Brl/+ and n=3 WT) were used to record ulnar strains during loading in order to assess the level of force required to induce strains that would produce linear microdamage in compression (estimated at 4000 micro strain) [16,18]. A 5 mm incision was made at the mid-diaphysis of the left forearm and the bones were further prepared using prescribed techniques [19] and commercial reagents (Vishay Micro-Measurements, U.S.A.) for the placement of strain gauges. Briefly, 100% ethanol followed by acetone was applied to the exposed ulna surface using a cotton tip applicator. A catalyst (Catalyst-C) was then applied followed by a drop of adhesive (M-Bond 200). Single element strain gauges (EA-06-015DJ-120, Vishay Micro-Measurements, U.S.A.) were placed longitudinally as close to the lateral surface as possible of the ulna of both Brl/+ and WT mice, near the mid-diaphysis. It has been confirmed experimentally [17], as well as through finite element modeling [20], that due to the geometry of the ulna, this area is the region of highest compressive strain and deformation during axial ulnar loading.

Cyclic loading was performed continuously, and several rounds of data were collected for each mouse and analyzed using linear regression in order to determine the load-strain relationship for both Brl/+ and WT genotypes. From these regressions it was noted that the loads that induced approximately 4000 micro strain were 2.71 ± 0.35 N for the Brl/+ and 4.92 ± 0.04 N for the WT mice.

Cyclic loading

A total of 12 cadaver mice (n=6 Brl/+ and n=6 WT) were used for cyclic loading. To induce microdamage, a similar loading construct and data recording protocol was used as described for in-situ strain gauging, with the exception that force and actuator displacement were recorded using Labview 7.1 software. Cyclic loading was performed in-situ in order to accurately simulate future in-vivo experiments involving sedated animals. The contralateral forelimbs were not loaded and were used as controls to assess baseline levels of microdamage in the bone matrix. Each specimen was allowed a 5 minute (300 cycles) period of dynamic pre-conditioning in order to mitigate the effects of soft tissue compliance and thus isolate and account for the effects of the actuator displacement to that of solely bone matrix degradation [20]. This time also afforded the opportunity to gradually ramp up the force amplitudes for each forearm to produce the required range of strains, as necessitated by the intrinsic differences in bone size between animals. Bones were then cyclically loaded for 30 min (1800 cycles) at force-controlled loads that produced approximately 4000 microstrain ($\mu\epsilon$) based on data collected in the in situ strain gauge experiments. Minimal compressive loads on the reversal phase of loading of approximately 2.45 N and 1.34 N for WT and Brl/+ mice, respectively, were used to ensure that platens remained in contact with the bone during the unloading segment. Post hoc calculations showed that WT mice displayed compressive strains ranging between a minimum of approximately $418.4 \mu\epsilon$ during the unloading segment and approximately $4010.8 \mu\epsilon$ (approximately 5 N) at maximum loading, whereas Brl/+ exhibited strains of 541.7 and $4039.1 \mu\epsilon$ (approximately 3 N) during unloading and loading segments, respectively. The displacement of the ulna during cyclic loading was recorded for each bone using a linear variable differential transformer (LVDT). Loss of stiffness for each bone was determined using Eq. (1), which was previously used by Bentolila et al. [16] as a surrogate for effective stiffness loss.

$$\text{Stiffness Loss} = \frac{L_o - L}{L_o} \quad (1)$$

where L_o is the initial length of bone and L is the final length of the bone. The initial length was always measured at the end of the dynamic pre-conditioning period (300 cycles) while L was measured when loading was complete. Both L_o and L were measured using the calibrated external LVDT during loading, while the forearm was still within the platens.

Microdamage quantification

After loading, the loaded (right) and unloaded (left) forelimbs were excised and stained with 1% basic fuchsin using an established protocol [21] and rinsed in 100% ethanol. Samples were subsequently embedded undecalcified in poly methyl methacrylate (PMMA) for histology. Using a Buehler Isomet diamond-blade saw, 400 μm sections were then cut transverse to the longitudinal axis of the ulna. These sections were mounted on a plastic microscope slide and polished to a final thickness of 100 μm . Sections were cover slipped and examined with a confocal microscope (Zeiss LSM 510-META Laser Scanning Confocal Microscope, Carl Zeiss MicroImaging, Inc., Thornwood, NY, U.S.A.) at 63 $\times$ magnification using a Helium Neon 1 (HeNe1) laser (543 nm) with a Texas red/rhodamine filter. Slides from the mid-diaphyses of each ulna were analyzed to quantify microdamage. Images were taken for the entire 100 μm thick cortical region and subsequently analyzed using the Zeiss LSM Image Browser (Version 4.2.0.121, Carl Zeiss Micro Imaging, Inc.) to quantify linear microdamage within the cortical region. Cracks that were positively labeled with basic fuchsin dye were deemed as damage-induced during loading. Diffuse damage was not assessed in this study. The following parameters were measured throughout the entire cortical bone region of each section: number of microcracks (#), bone cortical volume (B.Vol., mm^3), microcrack numerical density (Cr.Dn., $\#/\text{mm}^3$) and microcrack surface density (Cr.S.Dn., mm/mm^3).

Fracture toughness

To correlate microdamage with fracture toughness, femurs from each mouse were prepared and tested according to the techniques established by Ritchie [22]. This technique utilizes linear elastic fracture mechanics (LEFM) principles and a self-induced starter notch to measure the bone's resistance to fracture (fracture toughness). For regular mechanical testing of engineering materials, ASTM standards require that a sharp starter notch be the initiation point of fracture [23]. This starter notch is typically induced by a fatigue loading technique; however, due to the small geometry of mouse bones, this procedure is not optimum. Studies have shown that accurate fracture toughness data can be attained from razor sharpened micro-notches [24] and this technique has been deployed for small animal studies [22]. Using this technique will provide us with a true mechanical measure of the bone's inherent fracture toughness, without the viscoelastic influence of soft tissue.

The left femora of Brl/+ (n=6) and WT (n=6) were harvested, cleaned of soft tissue, and stored frozen at -20°C in gauze soaked in Lactated Ringer's Solution (LRS) until testing. Bones were machine-notched through the posterior cortex at the mid-shaft using a micro milling device with a cutting radius of 0.01 in. Notches were then sharpened using a custom rigged mounted razor blade, pasted with 1 μm diamond polish (Struers). Bone diameters were measured with electronic calipers and notch depths were made using a micro mill to approximately 1/3 of the bone diameter. This notching procedure typically produces a reproducible sharpened notch root radius of approximately 10 μm [22,24].

In preparation for three-point mechanical bend testing, the femoral heads were removed, while the condyles were left intact. This was done to maintain stability within the three-point bending apparatus. Femurs were thawed in LRS soaked gauze at room temperature to preserve moisture content until they were ready for testing. Specimens were loaded to failure in three-point bending at 0.001 mm/s using a servohydraulic testing machine equipped with a 220 N load cell (858 MiniBionixII Material Test System; MTS Systems Corporation, Eden Prairie, MN, USA) in accordance to ASTM standards [23,25] and other established protocol [22]. During three-point bending, the specimens were placed such that the posterior surface was in tension and the anterior surface was in compression with the top loading point oriented directly above the notch on the anterior surface. A span length of 4.5 mm was used in testing. Load and displacement values were recorded throughout the experiment using integrated Material Test System data acquisition software (858 MiniBionixII Material Test System; MTS Systems Corporation, Eden Prairie, MN, USA).

After mechanical testing, fractured bone specimens were fixed in 70% ethanol, defatted in toluene under vacuum for 24 h, and cleared of soft tissue with 2% potassium hydroxide for 12 h. The specimens were dehydrated by immersion in a graded series of alcohols (50%, 70%, 95% and 100%) and coated with carbon [10]. Specimens were imaged by SEM to determine the transition from stable to unstable crack growth (Fig. 1). Fracture toughness, K_{Ic} , was measured using solutions for circumferential through-wall cracks established for cylindrical pipes [22,26,27]. Both the maximum load and the crack instability techniques were used to determine fracture toughness values [22]. All measurements used in the LEFM calculations, such as specimen width and thickness, were taken from SEM images of fracture sites using the public domain software ImageJ (National Institutes of Health).

All measurements of thickness and radii were measured on the fracture surface of each specimen i.e. the non-notched surface as previously instructed by Ritchie [22]. Cortical thickness was taken as an average measurement of 6 different thicknesses measured at different points along the fracture surface. The centroid for each specimen was determined by drawing a line parallel to the notch that was half the bone's diameter. This line represented the major axis. Another axis that was rotated 90° to the first was also placed at half the bone diameter. This line represented the minor axis. The intersection of these perpendicular lines represented the centroid of the bone. In determining the stable to unstable transition region, the SEM was used in backscatter mode. The difference in morphology was used to determine the transition. The stable region is typically described as having a darker appearance with linear grooves, whereas the unstable region has a more spongy appearance. Half-crack angles (2θ) were determined by measuring the included angle (maximum load technique) which is formed from the centroid to the notched surface, and with the excluded angle (instability technique) which is formed from the centroid to the stable to unstable transition region.

Statistics

Effect of genotype on percent stiffness loss, crack numerical density, crack surface density, and fracture toughness values calculated using either the maximum load or instability technique were compared using a non-parametric Mann-Whitney test (GraphPad Prism 4.0) to account for small samples sizes. Linear regression between microdamage data and fracture toughness data was performed across genotypes and loading conditions. In all tests, significance was attributed to $p < 0.05$. All data is presented as mean $\pm$ SD.

Results

Brtl/± ulnae demonstrate lower stiffness loss during loading

Whole bone loss of stiffness for each forearm was measured via LVDT during cyclic loading according to Eq. (1). While Brtl/+ and WT ulnae were loaded with matching surface strains, Brtl/+ demonstrated a lower percentage stiffness loss ($1.77 \pm 0.84\%$) compared to WT ($3.56 \pm 1.29\%$) over the 35 minute load period (Fig. 2).

Brtl/± ulnae have more microdamage than WT

Non-loaded control limbs from Brtl/+ demonstrate significantly increased linear microcrack numerical density (217%) and microcrack surface density (354%) compared to their WT counterparts (Fig. 3A). In loaded limbs, Brtl/+ demonstrates a 220% increase in crack numerical density and 331% increases in crack surface density over WT (Fig. 3B). Linear microdamage was observed on the tensile and compressive (medial and lateral) sides of the ulnar cortices. While some cracks were oriented circumferentially, others ran through the cortex and spanned multiple lacuno-canalicular structures (Fig. 4).

Brtl/± femora display a moderate trend toward reduced fracture toughness compared to WT

Femora paired with loaded ulnae from within the same animal were tested for fracture toughness. During sample preparation and testing, 2 Brtl/+ and 2 WT specimens were lost due to errors in the notching process or due to premature fracture during three-point bending. Using the maximum load technique, which assesses fracture toughness based on the initial notch geometry and the maximum load encountered during three-point bending, WT femora displayed average K_{IC} values of 3.36 MPa m whereas Brtl/+ specimens had K_{IC} values of 2.73 MPa m ($p=0.486$) (Fig. 5A). The instability technique relies on identification of the transition between unstable and stable crack growth, and showed a more sensitive 35.4% difference in K_{IC} values between genotypes (WT 3.79 MPa m; Brtl/+ 2.44 MPa m) ($p=0.057$) (Fig. 5B). During crack instability calculations, four cases (3 WT and 1 Brtl/+) demonstrated half-crack angles that exceeded the range of values deemed valid ($\Theta/\pi=0.611$) for thick wall cylindrical pipe circumferential analysis ($\Theta/\pi=0.663, 0.645, 0.707, 0.756$) [22]. These represent specimens that are more ductile than the equation allows for accurate calculation. In those cases, by setting Θ/π to 0.611 for these specimens, an additional lower bound estimation of instability fracture toughness was estimated, resulting in a 31.4% difference in fracture toughness between Brtl/+ (2.44 MPa m) and WT (3.56 MPa m).

Microdamage is differentially regulated by fracture toughness in loaded vs. unloaded ulnae

The inability of maximum load fracture toughness values to be predictive of genotype precluded it from regression analysis. Fracture toughness measured by the instability technique was only predictive of crack numerical density and crack surface density when genotypes were pooled into separate regression models for loaded and unloaded ulnae (Fig. 6). Brtl/+ and WT ulnae showed a negative correlation between instability fracture toughness and crack surface density ($R=-0.85$, $p=0.01$) and instability fracture toughness and crack numerical density ($R=-0.72$, $p=0.04$). Brtl/+ and WT unloaded ulnar instability fracture toughness also showed a strong negative correlation with crack surface density ($R=-0.72$, $p=0.04$) and with crack numerical density ($R=-0.87$, $p=0.01$). While there was no significant difference between the regression slopes of unloaded and loaded crack numerical density ($p=0.4$, Fig. 6A), the relationship between instability fracture toughness and crack surface density varied depending on whether ulnae were loaded or unloaded ($p=0.02$, Fig. 6B).

Discussion

Currently, little is known about OI regarding the susceptibility of bones to produce and accumulate microdamage compared to bone with normal collagen. High propensity to form and accumulate microdamage may play a role in the inherent fragility and remodeling behavior seen in OI patients. This study utilized the *Brtl/+* mouse to investigate the correlation between propensity to form microdamage and the inherent fracture toughness of bone formed with an OI-causing mutation.

Brtl/+ ulnae subject to normal cage activity demonstrated a larger amount of cortical microdamage than WT controls. Furthermore, following 35 min of axial compressive loading matched for bone surface strains between genotypes, *Brtl/+* ulnae were more prone to damage than WT despite a greater resistance to whole-bone deformation. Femoral fracture toughness tests show a trend toward differences in K_C values between *Brtl/+* and WT, although these differences were not statistically significant. These fracture toughness values correlate well with levels of microdamage quantified in intact and mechanically-loaded limbs.

The moderate reductions in fracture toughness found in *Brtl/+* are consistent with reductions found in other mouse models of matrix insufficiency. Osteopontin deficient mice have reduced work to fracture (59%) and post-yield deformation (39.6%) compared to WT controls [28], and 8 week *OPN-/-* mice show a corresponding 30% decrease in instability fracture toughness compared to WT [29]. *Brtl/+* has shown similar reductions in work to failure (59%) and post-yield displacement (42%) [13] and a 35% decrease in fracture toughness in the present study.

The amount of energy that a material can withstand before the onset of a critical microcrack is an important parameter to consider in fracture mechanics [30,31]. In the present study, instability fracture toughness from *Brtl/+* showed a strong trend, but not statistically significant reduction compared to WT. This suggests that the lower work to failure and post-yield energy associated with *Brtl/+* may represent both an inability to prevent onset of critical microcracking, as well as a reduced resistance to crack growth toughness which was not directly measured in this study. Crack surface density appeared to be differentially regulated by instability fracture toughness between loaded and unloaded limbs in this study. As crack surface density can be indicative of crack growth, this suggests that crack growth, and potentially progression to failure may be differentially regulated based on whether the bone is loaded or not. Crack number, on the other hand, had a similar relationship to instability fracture toughness whether limbs were loaded or not. Crack numerical density can be an indicator of propensity for cracks to initiate, and these results suggest that the relationship between crack initiation and fracture toughness was not affected by whether the limb was loaded or unloaded. Together, these data suggest a bimodal response where fracture toughness governs crack initiation equally across a range of applied loads, but crack propagation becomes more susceptible to changes in fracture toughness with increased mechanical force.

Other OI models have implicated collagen integrity as a potential cause for OI bone's inability to withstand damage accumulation. *Oim* mice have demonstrated low work to failure and require fewer cycles to generate equivalent amounts of microdamage compared to WT counterparts [11]. The *Mov 13* mouse displays a purely quantitative *COL1A1* mutation with a 50% decrease in type 1 collagen content [32], and demonstrates a 61% decrease in post-yield displacement [10]. Furthermore, the cortical tissue components integral in the prevention of damage accumulation are severely compromised in *Mov 13*, leading to increased rate of damage accumulation and a decrease in the number of cycles to

failure when compared to WT [33]. It is evident that mutations involving type 1 collagen production have significant impact on the microdamage accumulation, fatigue strength and overall mechanical properties of bone.

Our in-situ strain gauge data shows that the *Brtl/+* ulnae required 45% less load to induce the prescribed 4000 $\mu\epsilon$. This is likely due to the geometric differences in bone sizes reported in other load bearing bones such as the femur [13]. X-ray images of ulnae demonstrated a small decrease in *Brtl/+* lengths compared to WT (1.8%) but a 25% decrease in diameter (data not shown). However, during loading, *Brtl/+* ulna showed a greater resistance to whole-bone deformation, likely due to previously observed increases in tissue mineral density associated with the collagen mutation, [34] thus leading to a less compliant bone compared to its wild type counterpart. This increase in tissue mineral density is in line with the more brittle phenotype, that has been previously displayed in *Brtl/+* [13] and in humans [1,35,36]. Our observance of higher linear microcracks in *Brtl/+* is supported by numerical probabilistic models, that show that higher mineralization in bone will facilitate the formation of linear microcracks [37]. This is further supported by experimental studies on dogs treated with bisphosphonates, which demonstrate bones had increased tissue mineralization with treatment but became more prone to a concomitant increase in microcracks and reduced energy absorption [38]. Despite *Brtl/+* specimens displaying a lower loss of stiffness, we still observe higher levels of microdamage. Previous studies have shown that there appears to be a difference in the surrounding bone matrix mineral composition between microdamaged areas and undamaged areas [39,40]. These studies provide evidence that there may be differences in the way energy from a microcrack is released to the surrounding bone matrix of *Brtl/+* and WT specimens, thus potentially explaining our high microdamage but low stiffness loss observations in *Brtl/+* specimens.

A significant amount of linear damage was observed at the tensile and compressive (medial and lateral) sides of the sample cortices and were oriented circumferentially. Previous studies of rat bone cyclically induced crack numerical densities ranging from 0.2 to 4.0/mm² and crack surface densities of approximately 0.2 mm/mm² [16,41]. In the present study, crack numerical densities ranged from 20 to 300/mm³, while surface densities were between 1 and 34 mm/mm³. The volumetric approach used in the present study accounts for these differences, as crack size and length were quantified through a 100 μm volume thickness, rather than a single section from the mid-span of the specimen. This volumetric approach minimizes the influence of surface polishing artifacts on the overall results, and quantifies damage through a larger segment of the tissue. As a means of verifying this approach, crack surface densities of several samples were analyzed on single optical slices. Results demonstrate that values from a single slice approach are within range of those observed by previous studies (data not shown).

Differences in fracture toughness were observed in our study, and were more noticeable using the instability technique. These differences reflect changes in half-crack angles between *Brtl/+* and WT, where, on average, *Brtl/+* specimens had smaller half-crack angles closer to the original starter notch, while WT specimens, with a more ductile fracture, had stable crack propagation further away from the starter notch. During these calculations, there were 4 cases (3 WT and 1 *Brtl/+*) where the half crack angles exceeded the range of angles deemed valid for accurate assessment of fracture toughness [22], indicating specimens that were more ductile than the equations can accurately calculate. To assess any potential error associated with these specimens, calculations were repeated using the maximum permissible half crack angle of 1.9195 rad as an upper limit estimate. As such, the determined value of K_{IC} for these re-calculated toughness values effectively represents a lower bound estimation of instability fracture toughness for ductile specimens. In this case, a 31.4% difference between *Brtl/+* and WT K_{IC} values remained (*Brtl/+* 2.44 MPa $\sqrt{\text{m}}$ and WT 3.56 MPa $\sqrt{\text{m}}$).

despite these estimated parameters. Furthermore, similar correlations between microdamage and instability fracture toughness were observed regardless of whether the original or re-calculated data was used, suggesting a minimal effect of these outliers on our conclusions. The lack of significance in our fracture toughness data may also suggest that a lower resistance to crack growth toughness, and not crack initiation as measured, may be the more prevalent underlying difference between *Brtl/+* and WT fracture toughness.

This study aimed to investigate the susceptibility of OI bone to microdamage accumulation and to explicate any role that microdamage may play in the inherent fragility and remodeling behavior seen in these patients. Although both osteoblast and osteoclast activities are increased in these patients, there is an imbalance in bone remodeling in favor of osteoclast activity [1], which ultimately leads to a compromised bone structure. It is unknown however as to what is the cause for this up-regulation in remodeling behavior seen in OI patients.

As microdamage levels increase in a healthy individual, the inherent remodeling processes of bone are activated for microdamage repair [42]. The increased remodeling activity seen in OI patients may be related to the extent of microdamage present in their bones, resulting from the compromised collagen inherent to the disease. *Brtl/+* mice demonstrate an increase in osteoclast number and function compared to WT [14], and the current findings demonstrate a correlative increase in propensity to generate and accumulate microdamage in the bone. While the cellular asynchrony favoring osteoclast formation and activity in *Brtl/+* is a result of an increase in osteoclast precursors in *Brtl/+* marrow [14], the RANKL/OPG ratio is unchanged and the factors regulating increased osteoclasts have yet to be described. Further *in vivo* loading experiments will determine whether generation and accumulation of skeletal microdamage in *Brtl/+* are responsible for these increased remodeling rates.

A widespread treatment for the high turnover and low bone mass condition of OI is bisphosphonate therapy. Bisphosphonates function by reducing bone turnover and increasing skeletal mass, but do not target the underlying genetic abnormalities associated with the disease [34]. While the intention for OI treatment with bisphosphonates is to increase skeletal mass, they may concomitantly prevent targeted repair from occurring. It has been shown that downregulation of bone remodeling with bisphosphonate therapy results in an increased accumulation of skeletal microdamage in other models [43–46], subsequently resulting in a reduction of skeletal mechanical integrity [43,45–47]. The long-term implications for bisphosphonate therapy in a low-fracture toughness skeleton prone to the generation of microdamage have not yet been described.

In conclusion, our studies have shown that the *Brtl/+* mouse model for OI is more susceptible to microdamage accumulation than age-matched WT. These data suggest the possibility that OI patients may engender larger degrees of damage due to the inherent collagen mutation. With the increase in damage comes an additional risk of lowered resistance to bone fracture. Increased microdamage and reduced fracture toughness observed in the *Brtl/+* model for osteogenesis imperfecta suggest that microdamage may play a significant role in contributing to bone fragility and increased remodeling associated with OI. These findings have strong clinical implications for explaining increased fragility and remodeling activity in OI patients.

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Fig. 1. SEM image showing the notch, stable crack growth and unstable crack growth region used to determine the half-crack angles for assessing the instability fracture toughness.

Fig. 2.

Average stiffness loss for each phenotype after 5 min of 1 Hz cyclic pre-conditioning loading and 30 min of 1 Hz cyclic loading. Brl/+ specimens display lower loss of stiffness (1.77%) when compared to their WT counterparts (3.56%) under cyclic loading. * $p < 0.05$.

Fig. 3.

Summary data quantifying the amount of microdamage seen in both loaded and unloaded Btl/+ and WT specimens. The crack numerical density (Cr.Dn), which measures the number of individual linear microcracks along the specimen surface area for both loaded and unloaded Btl/+ and WT specimens, can be seen in (A). The crack surface density (Cr.S.Dn) which measures the amount of damaged tissue across the specimen surface area, can be seen in (B). * $p < 0.05$ vs. WT within loading condition.

Fig. 4. Confocal photomicrographs of basic fuchsin stained cross sections obtained from the ulna mid-diaphysis of an unloaded Brl/+ specimen (A) and a loaded Brl/+ specimen (B). Linear microcracks in the cortex are stained with basic fuchsin. Scale bar 20 μm with a photomicrograph field width of 128 μm .

Fig. 5.

Graph showing the average fracture toughness values for WT and BRTL/+ specimens tested using the maximum load (A) and instability techniques (B) described by Ritchie [22]. There was an 18.6% difference noted with the maximum load technique ($p=0.486$) whereas as 35.4% difference was noted with the instability technique ($p=0.057$).